SELECTIVE EFFECT OF ORGANOPHOSPHATE INSECTICIDES ON METABOLIC ACTIVITIES AND AFLATOXINS BIOSYNTHESIS BY TWO *ASPERGILLUS* SPP.

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ABSTRACT - In liquid cultures treated with three organophosphate insecticides (Dimethoate, Malathion, and Selecron) at rates 10, 50, 100, 250 and 500 mg/L active ingredient, mycelial production by *Aspergillus flavus* IMI 89717 was significantly promoted with most treatments and inhibited only at 500 mg/L level. Mycelial dry weight of *A. parasiticus* var. *globosus* IMI 120920 was significantly delayed by all used doses. Respiration of *A. flavus* was decreased ▬ the higher rates of Dimethoate and Malathion but the effect of Selecron was unclear. CO_2 production by *A. parasiticus* was promoted in all cases. The pattern for depletion of total fatty acids with Malathion was similar to that for aflatoxins while ▬ inverse relationship was recorded between fatty acids and aflatoxins biosynthesis with Dimethoate and Selecron by both toxigenic aspergilli. On sorghum treated with the three insecticides, the highest application rate (5000 mg/kg) suppressed the production of aflatoxins B_1, B_2, G_1 and G_2 by the two tested aspergilli.

RÉSUMÉ - En milieu liquide, l'ajout de trois insecticides organophosphorés (Dimethoate, Malathion, Selectron), aux concentrations de 10, 50, 100, 250 et 500 mg/l augmente la croissance mycélienne d'*Aspergillus flavus* IMI 89717 pour la plupart des concentrations et n'inhibe la croissance qu'à partir de 500mg/l. La croissance mycélienne d'*Aspergillus parasiticus* var. *globosus* est par contre inhibée aux doses d'insecticides utilisées. L'activité respiratoire d'*Aspergillus flavus* diminue aux doses élevées de Dimethoate et de Malathion, alors que l'action du Selectron est moins nette. La production de CO_2 par *Aspergillus parasiticus* est stimulée dans tous les cas. En présence de Malathion, les taux d'acides gras et d'aflatoxines des deux espèces étudiées diminuent, contrairement à ce qui a été observé en présence de Dimethoate ou de Selectron, où leurs variations sont inverses. Sur le Sorgho, le traitement par les trois insecticides aux concentrations de 5000 mg/kg inhibe la production d'aflatoxines B_1, B_2, G_1 et G_2 pour les deux *Aspergillus* étudiés.

KEY WORDS : aflatoxins, fatty acids, insecticides, respiration.

INTRODUCTION

In warm and tropical countries, stored grain insects such as *Sitophilus* increase the moisture of the grains and can thus initiate hot spots. Many storage fungi including *Aspergillus* spp. have been isolated from stored grain insects, thus proving that insects act as vectors for the fungi (Wallace, 1973). Aflatoxins ▬ a group of highly toxic secondary metabolites produced by the fungi, *Aspergillus flavus* and *A. parasiticus* in

corn, cotton seed, peanuts and other commodities in the field and storage. Aside from their much feared carcinogenic potential, aflatoxins cause serious economic loss to the nation's agriculture every year.

Organophosphate insecticides are one of the most important groups of modern pesticides that are usually used in agricultural practice, to resist the harmful insects that attack crops, due to their high insecticidal activity, the broad spectrum and rapid action on pests, and to their decomposition with formation of products non toxic to human and animals (Gruzdyev et al., 1983).

In past reviews it was found that organophosphate insecticides strongly inhibited aflatoxin production by *Aspergillus* spp. (Rao & Harein, 1972, 1973; Hsieh, 1973; Schroeder et al., 1974; Draughon & Ayres, 1978, 1979, 1981; Draughon et al., 1983). On the other hand, Draughon (1983) found that some organophosphate insecticides such as Dursban and Nellite, increased aflatoxin production by *A. flavus* in liquid medium. Also, she warned that certain insecticides could stimulate growth and aflatoxin production by *Aspergillus* spp. on crops. Also respiration and mycelial growth of fungi was found to be affected by insecticides application (Anderson, 1978).

Our purpose was to determine the effects of three extensively used organophosphate insecticides on growth, respiration and synthesis of fatty acids in liquid medium by toxigenic moulds. Also, their effect on aflatoxins production in liquid medium and on natural substrate (sorghum grains), was investigated.

MATERIALS AND METHODS

Microorganisms:

Aspergillus flavus IMI 89717 and *A. parasiticus* var. *globosus* IMI 120920 were used throughout this study. The two moulds were maintained on potato dextrose agar (PDA) slants.

Medium:

To measure aflatoxin production, fatty acids synthesis, growth and CO_2 evolution, Czapek's Dox medium was used. Aflatoxin production was also carried out on sorghum grains.

Insecticides:

Three organophosphate insecticides were used in this investigation, Dimethoate [0,0-dimethyl s-(2-(methylamino)-2-oxoethyl) phosphorodithioate], Kafr El-Zayat; Malathion [0,0- dimethyl -s -(1,2-dicarbethoxyethyl) phosphorodithioate], El-Naser Chemicals Co. and Selecron [0-(4-bromo-2-chloro-phenyl) 0-ethyl S-n-propyl phosphorothioate], Ciba Geigy.

Procedure:

The Dox medium was dispended in 250 ml Erlenmeyer conical flasks with 50ml in each, flasks were autoclaved at 121°C for 15 min. Insecticides were sterilized through membrane filter and added to cooled autoclaved media to give concentration of 10, 50, 100, 250 and 500 mg/L active ingredient. Flasks used as blanks received sterilized water equivalent to volumes of insecticides used. After gentil shaken, control and treated flasks were inoculated with 1ml of spore suspension of 7 days old agar slant.

Lots of 50g of sorghum grains were sterilized by shaking in 5% NaOCl solution for 5 min and rinsing in three changes of sterile distilled water. Thereafter, the samples were placed in sterile polyethylene bags and thoroughly mixed with different doses of the insecticides. Each bag was inoculated by 1 ml of ▪ heavy spore suspension. Sterile distilled water was added to raise the moisture content of the grains to 25%. The liquid and sorghum grains cultures were incubated at 28°C for 7 and 14 days, respectively, before measuring fungal activities.

Mycelial growth determination:

Mycelia of 7 days old cultures were harvested by filtration through Whatman filter paper n° 1 under lower pressure vacuum then dried at 80°C till constant dry weight.

Measuring of mycelial respiration:

The modified substrate-induced respiration technique (Cheng & Coleman, 1989) was used for measuring mycelial respiration. Two conical flasks for control and treatments, containing 7 days old cultures, were flushed with continuous CO_2-free air. The CO_2 evolved from the cultures was carried by the air current to the final CO_2 trap (50ml of 0.5 M NaOH). After 10 hours exposure, the final traps were removed and mixed with excess of $BaCl_2$ solution to precipitate carbonate. The NaOH in the final trap is then titrated with 0.25 M HCl using phenolphthalein ▪ an indicator.

Aflatoxin analysis:

For this purpose cultures (filtrate + mycelium) and sorghum grains cultures of 7 and 14 days old, respectively, were extracted with 100 ml chloroform. The extract was then evaporated till dryness on a rotary evaporator. Aflatoxin residue was dissolved in chloroform and separated by thin-layer chromatography on Silica Gel 60-coated plates, using chloroform-acetone (9:1) as the developing solvent. The spots of aflatoxins B_1, B_2, G_1 and G_2 were removed from the plate, eluted with methanol and estimated spectrophotometrically (Nabney & Nesbitt, 1965). Aflatoxins B_1 and B_2 were estimated together as aflatoxin B, and G_1 and G_2 as aflatoxin G, using extinction coefficients for aflatoxins B_1 and G_1, respectively.

Fatty acids determination:

The total fatty acids were determined by the phosphovanillin method of Zöllner & Kirsch (1962).

RESULTS AND DISCUSSION

Regarding to results presented in table 1, mycelial growth and mycelial respiration of the two tested moulds showed different responses to insecticides application.

Mycelial biomass of *A. flavus* was significantly increased with most doses of Dimethoate, Malathion and Selecron whereas the highest dose (500 mg/L) has inhibitory effect. In case of *A. parasiticus*, the mycelial dry weight was significantly decreased, compared with the control, with all used doses of insecticides and the inhibition was more pronounced in case of Selecron than Dimethoate and Malathion. The selective effect of organophosphate insecticides on growth of fungi was discussed before by some authors. Cowley & Lichtenstein (1970) reported that Phorate at 1 and 2 mg/L stimulated the growth of *A. fumigatus*. Also, Hasan (1988) found that Actellic

organophosphate insecticides	Concn (mg a.i./L)[a]	A. flavus		A. parasitivus	
		Dry wt	CO_2 evolution	Dry wt	CO_2 evolution
	0	250	48.0	424	24.9
Dimethoate	10	296*	70.4*	318*	33.2
	50	292*	48.0	334*	31.6
	100	278*	30.4*	254*	41.6*
	250	300*	30.4*	280*	41.1*
	500	150*	35.2*	224*	36.4*
Malathion	10	314*	48.0	308*	65.5*
	50	326*	54.6	348*	99.3*
	100	312*	61.2*	286*	53.7*
	250	280*	39.4*	330*	94.5*
	500	242	29.5*	328*	36.6*
Selecron	10	344*	61.2*	266*	75.8*
	50	278*	48.0	266*	166.0*
	100	268*	48.0	244*	161.3*
	250	232	59.3*	216*	55.5*
	500	200*	52.8	172*	50.2*

Table 1: Effect of organophosphate insecticides on mycelial growth (mg/50ml medium) and CO_2 evolution (mg/g dry wt/24h) of A. flavus ■ 89717 and A. parasiticus var. globosus IMI 120920.

* Means significant difference compared to the control ■ 5% level.

proved to be promotive to the mycelial growth of Curvularia lunata at 10 mg/L. Increasing growth of fungi in cultures treated with insecticides was regarded as a result of their ability to degrade insecticides (Anderson & Lichtenstein, 1972). On the other hand, the adverse effect of insecticides on growth of fungi was documented. In this respect, Cunninghamella echinulata was completely eliminated by 32.7 mg/L Curacron (Abdel-Kader et al., 1981). Also, Abdel-Kader et al. (1984) studied the effect of incorporation of Phosphamidon into Czapek's liquid media on the growth of fungi. They reported that the three used doses (1,4 and 8 mg/L) inhibited the growth of Penicillium corylophilum. Other results came in agreement with our finding. They were obtained by Abdel-Mallek (1984) and Shonquir (1989). El-Abyad et al. (1988) referred to the inhibition of growth of two Fusarium wilt fungi due to decrease in sugar uptake.

Organoph- osphate insecticides	Concn (ppm) a.i.)	A. flavus					A. parasiticus				
		Total fatty acids (as oleic acid)	Aflatoxins				Total fatty acids (as oleic acid)	Aflatoxins			
			B_1+B_2	inhi- bition %	G_1+G_2	inhi- bition %		B_1+B_2	inhi- bition %	G_1+G_2	inhi- bition %
	0	21.4	290	0.0	380	0.0	19.6	310	0.0	400	0.0
Dimethoate	10	22.7	295	0.0	390	0.0	20.1	310	0.0	400	0.0
	50	29.8*	315	0.0	422	0.0	20.1	310	0.0	400	0.0
	100	27.2*	350*	0.0	456*	0.0	20.5	280	9.7	360	10.0
	250	28.2*	232*	20.0	360*	5.3	20.9	276*	11.0	320*	20.0
	500	35.8*	87*	70.0	228*	40.0	21.8	186*	40.0	240*	40.0
Malathion	10	19.8	290	0.0	380	0.0	19.7	310	0.0	400	0.0
	50	19.9	280	3.5	360	5.3	19.6	372*	0.0	460	0.0
	100	19.7	270	6.9	340	10.5	19.3	356*	0.0	480*	0.0
	250	17.6*	58*	80.0	76*	80.0	18.9	124*	60.0	166*	48.5
	500	18.5*	15*	94.8	19*	95.0	16.4*	66*	78.7	86*	78.5
Selecron	10	21.7	230*	20.7	370	2.6	29.7*	68*	78.1	80*	80.0
	50	20.3	174*	40.0	230*	39.5	31.3	62*	80.0	80*	80.0
	100	27.5*	116*	60.0	152*	60.0	24.7*	58*	81.3	70*	82.5
	250	33.7*	62*	78.6	148*	61.1	26.6*	42*	86.5	40*	90.0
	500	36.4*	54*	81.4	142*	62.6	32.6*	31*	90.0	34*	91.5

Table 2: Effect of organophosphate insecticides on total fatty acids content (mg/g dry wt) and production of aflatoxins (µg/g dry wt) by A. *flavus* IMI 89717 and A. *parasiticus* var. *globosus* IMI 120920.

* Means significant difference compared to the control at 5% level.

Mycelial respiration of *A. parasiticus* occurs in opposite manner to mycelial dry weight but respiration of *A. flavus* was inhibited with the higher rates of Dimethoate and Malathion whereas slightly influenced by Selecron. Disturbance of respiration under stress conditions appear to be a general phenomenon. However, insecticides seem likely stimulate respiration (Anderson, 1978). Increasing rate of respiration by toxicants was recorded by McCallan et al. (1954). O_2 uptake by *A. fumigatus, Cunninghamella echinulata* and *P. funiculosum* was stimulated by the organophosphate insecticide Dimethoate when applied at rate of 13.5 and 67.5 mg/L (Shonquir, 1989). In recent investigation, Abdel-Basset et al. (1992) reported that respiration of three mesophilic fungi was fluctuated by the insecticide Selecron but they found that the increase in CO_2 evolution was more pronounced. The increase in respiration rate by toxicants may possibly be due to increase in membrane permeability to substrate as indicated by Kurtz et al. (1982). Another explanation for increasing rate of respiration is the stimulation of metabolic activity as well ■ uncoupling of oxidative phosphorylation (Anderson, 1978). On the other hand, inhibition of respiration by pesticides application could be due to inhibition of oxidative phosphorylation and ATPase activity as concluded by Tam & Trevors (1981).

The effect of selected organophosphate insecticides on total fatty acids contents and aflatoxins production by the two toxigenic moulds in liquid medium was variable (Table 2). Fatty acids synthesis by the two fungi was delayed with Malathion. Kutzner & Buchenauer (1986) have shown that the Triazole fungicides treatment caused an appreciable reduction in mycelial triglyceride contents of *Fusarium moniliforme*. On the other hand, Dimethoate and Selecron caused an increase in fatty acids content of *A. flavus* and *A. parasiticus*. These results agree with that reported by Detroy & Hesseltine (1969). Also Weete & Wise (1987) found that treatment of *A. ochraceus* with Triazoles caused accumulation of free fatty acids and increased linoleic acid.

Production of aflatoxins B_1, B_2, G_1 and G_2 in liquid medium was inhibited by the three insecticides at 250 and 500 mg/L levels and the inhibition was more pronounced with Selecron followed by Malathion and Dimethoate (Table 2). Toxins production by *A. flavus* was promoted with 100 mg/L Dimethoate. The stimulatory effect on toxins accumulation in case of *A. parasiticus* was confined to 50 and 100 mg/L Malathion. Induction of aflatoxin synthesis has been observed perviously in cultures of *A. flavus* treated by the organophosphate insecticides Dursban and Nellite (Draughon, 1983). On the other hand, the higher concentration (500 mg/L) of Dimethoate significantly inhibited aflatoxins B_1 and B_2 by 70% and G_1 and G_2 by 40% in *A. flavus* and all aflatoxins by 40% in *A. parasiticus*. Also, production of aflatoxins B_1, B_2, G_1 and G_2 in both aspergilli was significantly inhibited at 250 mg/L of Malathion. Increasing the concentration of Malathion to 500 mg/L increased inhibition of aflatoxins production. Also, all aflatoxins were inhibited by all Selecron concentrations, which represents the most effective insecticide. The inhibition of aflatoxins synthesis by organophosphate insecticides was reported before by Draughon & Ayres (1978, 1979, 1981). They found that Naled, Diazinon, Dyfonate and Malathion significantly inhibited aflatoxin production at 100 mg/L. Also, Malathion inhibited production of citrinin and patulin by 96 and 42%, respectively.

The pattern for depletion of total fatty acids with Malathion was similar to that for aflatoxins, but an inverse relationship was recorded with Dimethoate and Selecron in both toxigenic aspergilli. Several authors recorded a relationship between lipid metabolism and aflatoxins biosynthesis. Detroy & Hesseltine (1969) reported an inverse relationship between fatty acids and aflatoxin formation. Shih & Marth (1974), on the other hand, found that the pattern for formation and depletion of total lipids was simi-

organophosphate insecticides	concn (mg a.i./kg)	A. flavus				A. parasiticus			
		aflatoxin B_1+B_2	Inhibition %	Aflatoxin G_1+G_2	Inhibition %	Aflatoxin B_1+B_2	Inhibition %	Aflatoxin G_1+G_2	Inhibition %
		430	-	230	-	680	-	350	-
Dimethoate	1000	810*	-	650*	-	750*	-	490*	-
	5000	220*	48.8	115*	50.0	205*	69.9	58*	83.4
Malathion	1000	420	2.3	320*	-	540	20.6	230*	34.3
	5000	280*	34.9	95*	58.7	270*	60.3	110*	68.6
Selecron	1000	640*	-	420*	-	340*	50.0	170*	51.4
	5000	330*	23.3	155*	32.6	130*	30.9	90*	74.3

Table 3: Effect of organophosphate insecticides on aflatoxin production (µg/kg grains) by A. flavus IMI 89717 and A. parasiticus var. globosus IMI 120920 on sorghum grains.
* Means significant difference compared to the control at 5% level. a.i. = active ingredient insecticide.

lar to that for aflatoxins, whereas acetate was the common precursor for both aflatoxins and lipid synthesis. Also, Rao & Harein (1973) explained the inhibition of aflatoxin biosynthesis due to interference with the acetate-malonate pathway which seems likely to be ■ common route in the biosynthesis of secondary metabolites of fungi.

Influence of insecticides on aflatoxin production by the the toxigenic aspergilli was also carried out on sorghum grains at 1000 and 5000 mg active ingredient /kg (table 3). The low dose of application induced promotive effect on the synthesis of aflatoxin especially by A. flavus. Stimulation of aflatoxin formation was previously recorded when some pesticides were used for prevention of these toxins (Draughon, 1983; Badii & Moss, 1988; Hasan, 1991). However, the high application rate (5000 mg/kg) inhibited aflatoxin B_1 and B_2 by 23.3-48.8% and G_1 and G_2 by 32.6-58.7% in A. flavus. Also inhibited aflatoxin B_1 and B_2 by 60.3-80.9% and G_1 and G_2 by 68.6-83.4% in A. parasiticus. In this respect Draughon et al. (1983) found that Naled (organophosphate) at 100 ppm inhibited aflatoxin production in culture medium, but did not affectively reduce aflatoxin production when applied to corn. However Rao & Harein (1972, 1973) and Shroeder et al. (1974) reported that Dichlorvos (organophosphate) at 20 ppm inhibited aflatoxin production in rice, corn, wheat and peanuts. Further work is needed to determine the activity of modern pesticides against different mycotoxin production in foods, feeds or other materials.

CONCLUSION

It is worth mentioning that the organophosphate insecticides promotive the synthesis of aflatoxin by *A. flavus* or/and *A. parasiticus* at 100 mg/L in liquid medium and 1000 mg/kg on sorghum grains. However, they restricted toxin production at 250 and 500 mg/L in liquid medium and 5000 mg/kg on sorghum grains. Selecron had most pronounced effect than Dimethoate and Malathion. Our finding is helpful for prevention purpose of seed stocks for contamination by aflatoxigenic moulds if the insecticides are used in sufficient rates.

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